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(54) Title: TELOMERASE-SPECIFIC CANCER VACCINE

(57) Abstract

Telomerase-specific T-cell antigens are provided, which are useful in generating T-cell responses against telomerase. Formulations of telomerase antigens as vaccines are useful in treating and preventing cancer, using *in vivo* or *ex vivo* techniques.

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TELOMERASE-SPECIFIC CANCER VACCINE

BACKGROUND OF THE INVENTION

Telomeres, the DNA at the chromosome ends, are made up of simple tandem
repeats. In most somatic cells, telomere sequences are lost during DNA replication due to the
need of DNA-dependent DNA polymerases for an RNA primer annealed to the template
strand. Because the RNA primer cannot anneal beyond the 5' end of the DNA strand, each

10 time a cell's DNA replicates, short bits of telomeric DNA are lost with each generation. Cells
displaying such telomeric shortening go into senescence after a fixed number of population
doublings, and senescence correlates directly with the erosion of telomeres to a critical
minimum length.

Not all cells undergo such loss, however. While normal human somatic cells

15 lose telomeric repeats with each cycle of cell division, human germline and, significantly,
cancer cells maintain a constant number of telomeric repeats. Telomere length is maintained
in these cells by the action of telomerase, a ribonucleoprotein enzyme that uses a short
endogenous RNA as a template for telomere addition. In fact, cancer cells express high levels
of telomerase, whereas somatic cells express little, if any.

20 Because normal somatic cells do not appear to express or require telomerase,
whereas cancer cells express high levels of telomerase, the telomerase enzyme presents an
attractive therapeutic target. Due to the fact that telomerase is a normal "self" antigen,
however, conventional vaccination strategies are unavailable. Thus, the focus of telomerase-
based therapeutics has been enzyme inhibitors of various sorts, rather than vaccine-based

25 approaches.

A need exists, therefore, for new telomerase-based therapeutic approaches.
This need extends to vaccines, based on telomerase antigens.

SUMMARY OF THE INVENTION

It is, therefore, an object of the invention to provide antigens that are useful in
generating an immune response against telomerase. According to this object of the invention,
telomerase antigens are provided that are capable of marshalling the immune system against

5 telomerase-expressing cells. In one embodiment, telomerase antigens are provided, which are
based on peptide sequences of the protein portion of telomerase. In another embodiment,
telomerase antigens are provided as nucleic acids that are capable of being used to express
peptide-based telomerase antigens.

It is another object of the invention to provide vaccine compositions which

10 include at least one telomerase antigen. Thus, in one aspect, the invention provides vaccine
compositions containing telomerase peptide antigens. In another aspect, polynucleotides are
provided, which encode protein-based telomerase antigens.

It is still another object of the invention to provide methods of treating or
preventing cancer. According to this object, the telomerase antigens of the invention may be

15 directly administered in beneficial amounts to a patient. Also according to this object, the
present telomerase antigens may be administered to a patient encoded in a nucleic acid. This
object is also met by ex vivo methods that involve contacting a cell with a telomerase antigen,
and administering that cell to a patient. In one aspect, the contacted cell may be an antigen
presenting cell, which may be used to generate a primed T-cell ex vivo, at which time the

20 primed T-cell may be administered to a patient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows fluorescence activated cell sorting (FACS) analysis of T-cells activated by
telomerase-specific antigens. Panels A and B are negative and positive controls, respectively,
and panels C, D and E are antigen candidates.

DETAILED DESCRIPTION

The present invention relates to a telomerase-specific vaccines, which are useful generating telomerase-directed activated T-cells. More particularly the inventive vaccines are useful in generating antigen-specific major histocompatability- (MHC-)

5 restricted T-cell responses against telomerase presented by antigen presenting cells. It is believed that the present vaccines act to relieve the tolerance or anergy induced through self-tolerance mechanisms to telomerase in normal individuals. Since telomerase represents a cancer-specific therapeutic target, in one embodiment, the vaccines of the invention are useful in the treatment or prevention of a variety of cancers.

10 **A. Definitions**

As used in this specification, an "activated T-cell" is one that is in the following phases of the cell cycle: the G_1 phase, the S phase, the G_2 phase or the M (mitosis) phase. Thus, an "activated T-cell" is undergoing mitosis and/or cell division. An activated T-cell may be a T helper (T_H) cell or a cytotoxic T-cell (cytotoxic T lymphocyte (CTL or T_C)).

15 Activation of a naive T-cell may be initiated by exposure of such a cell to an antigen-presenting cell (APC) (which contains antigen/MHC complexes) and to a molecule such as IL-1, IL-2, IL-12, IL-13, γ-IFN, and similar lymphokines. The antigen/MHC complex interacts with a receptor on the surface of the T-cell (T-cell receptor (TCR)). Golub et al., eds. IMMUNOLOGY: A SYNTHESIS, Chapter 2: "The T-cell Receptor" (1991).

20 As used in this specification, "priming" is used to mean exposing an animal (including a human) or cultured cells to antigen, in a manner that results in activation and/or memory. The generation of CD4⁻ and CD8⁻ T-cell responses against a target antigen is usually dependent upon in vivo priming, either through natural infection or through deliberate immunization.

25 As used in this specification, a "naive" T-cell is one that has not been exposed to foreign antigen (non-autologous) antigen or one that has not been exposed to cryptic autologous antigen. A "naive" T-cell is sometimes referred to as an "unprimed" T-cell. The

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skilled artisan will recognize that a "resting" cell is in the G_0 phase of the cell cycle and hence is not dividing or undergoing mitosis. The skilled artisan will also recognize that an "anergic" T-cell is one that is unable to function properly: i.e. such as a cell that lacks the ability to mediate the normal immune response. T-cells from diseased patients may contain T-cells that

5 have been primed, but are anergic.

As used in this specification "memory T-cells," also known as "memory phenotype" T-cells, is used to designate a class of T-cells that have previously encountered a peptide antigen but are now resting and are capable of being activated. Memory T-cells are T-cells which have been exposed to antigen and then survive for extended periods in the body

10 without the presence of stimulating antigen. However, these memory T-cells respond to "recall" antigens. In general, memory T-cells are more responsive to a "recall" antigen, when compared with the naive T-cell response to peptide antigen. Memory cells can be recognized by the presence of certain cell-surface antigens, such as CD45R0, CD58, CD11α, CD29, CD44 and CD26, which are markers for differentiated T-cells.

15 As used in this specification, an "telomerase-specific" T-cell response is a T-cell response (for example, proliferative, cytotoxic and/or cytokine secretion) to telomerase antigenic stimulus, for example a peptide, which is not evident with other stimuli, such as peptides with different amino acid sequences (control peptides). The responsiveness of the T-cell is measured by assessing the appearance of cell surface molecules that are characteristic

20 of T-cell activation, including, but not limited to CD25 and CD69. Such assays are known in the art.

The term "treating" in its various grammatical forms in relation to the present invention refers to preventing, curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a disease state, disease progression, disease

25 causative agent or other abnormal condition.

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B. Telomerase Antigens

1. Generally Useful Antigens

Telomerase antigens according to the invention share the characteristic ability to generate a specific T-cell response. This response may be either class I- or class II-specific. In one aspect, this response is MHC class I-specific, and will comprise antigen- and MHC-restricted cytotoxicity. Class I molecules include HLA-A, HLA-B and HLA-C. Thus, preferred antigens bind class I molecules, e.g., HLA-A1, HLA-A2, HLA-A3 or HLA-A11. More preferred antigens bind all class I molecules. In contrast, where class II-specific (e.g., helper functions) responses are desired, class-II-binding antigens will be used. Class II molecules include HLA-DR, HLA-DQ and HLA-DP. Useful antigens can be determined as set out below.

Telomerase antigens are typically derived from the sequence of the protein portion of telomerase, which is disclosed in U.S. Patent No. 5,837,857 (1998) and at GenBank Accession Nos. AF015950 and AF018167, which sequences are hereby incorporated by reference. They may be made, for example, by proteolytic digestions of the telomerase protein and/or by recombinant DNA means. Generally, the relatively short peptide versions will be prepared by synthetic means.

Although telomerase antigens according to the invention are not limited by size, and they may be a portion or even all of the telomerase protein, they are usually small peptide antigens. A small size is preferred, due to ease of manufacture and greater specificity. Accordingly, unless they are multimeric (i.e., multiple copies of the same epitope) most telomere antigens will be less than about 50 amino acids in length. Preferred antigens are less than about 25 amino acids in length, with other preferred antigens being between about 8 to about 12 amino acids long, although sequences as short as 6 or 7 amino acids are contemplated. Nine-mers are typical of class I antigens, since they usually retain the requisite functional character; they include Ile-Leu-Ala-Lys-Phe-Leu-His-Trp-Leu (ILAKFLHWL) as a preferred species.

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Variants of telomerase antigens are also contemplated. It is only important that any variants retain the functional characteristics of a telomerase antigen: (1) the ability to bind an MHC molecule, e.g., HLA-A2, and (2) the ability to induce a telomerase specific T-cell response. Amino acid substitutions, i.e. "conservative substitutions" that yield "conservative variants," may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

For example: (a) nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b) polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (c) positively charged (basic) amino acids include arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Substitutions typically may be made within groups (a)-(d). In addition, glycine and proline may be substituted for one another based on their relatively small sizes and lack of side-chains.

Similarly, certain amino acids, such as alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine and lysine are more commonly found in α-helices, while valine, isoleucine, phenylalanine, tyrosine, tryptophan and threonine are more commonly found in β-pleated sheets. Glycine, serine, aspartic acid, asparagine, and proline are commonly found in turns. The importance of substitution groups based on structure, of course, increases with the length of the antigen.

Some preferred conservative substitutions may be made among the following groups: (i) S and T; (ii) P and G; and (iii) A, V, L and I. Given the known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist readily can construct DNAs encoding the conservative amino acid variants. Of course, smaller variants may be synthesized. One such genus of conservative HLA-A2-binding variants includes peptides of the structure: (A/V/L/I)(A/V/L/I)(A/V/L/I)(A/V-L/I)KF(A/V/L/I)HW(A/V/L/I). Thus, some preferred conservative variants include LLAKFLHWL, ILAKFLHWL, IIAKFLHWI, IIAKFLHWL,

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ILAKFLHWI, ILARFLHWL, and permutations thereof, so long as the requisite functional characteristics are retained.

Moreover, one or more of the amino acids of the foregoing HLA-A2-binding peptides may be replaced with glycine. Parker et al., J. Immunol. 149:3580-87 (1992)

5 disclose that up to six amino acids in a nine-mer may be replaced with glycine; thus, GLFGGGGGV can bind HLA-A2. The only real conservation observed in 9-mer HLA-A2-binding peptides was an Ile or a Leu at about position 2 (counting N- to C-terminal) and a Val or a Leu at about position 9. Some simple variants, therefore, include GLAKFLHWL, ILAGFLHWL, ILAKGLHWL, ILAKFGHWL, ILAKFLGWL and ILAKFLHGL, subject to

10 the presence of the requisite functional characteristics.

An important source for guidance in regard to designing class I and class II antigens, and in making conservative substitutions is Rammensee et al., Immunogenetics 41:178-228 (1995), which is hereby incorporated by reference in its entirety. As indicated in the Rammensee reference, the motifs for both class I and class II molecules have certain

15 "anchor" residues, that retain high degrees of conservation. For instance, HLA-A0201 (an HLA-A2 molecule), which is the molecule that the telomerase peptide ILAKFLHWL was designed to bind (and does bind), has anchor residues at positions 2 and 9, corresponding to the conservative positions noted above. This molecule also has an "auxiliary" position at 6, the relative conservation of which is important, but less so than the anchor residues. Thus,

20 using the general guidance of Rammensee, the artisan will appreciate that, while the anchor residues and auxiliary residues are relatively conserved in HLA binding, the remainder of the antigen can vary widely, and is probably responsible for the particular antigenic character of the antigen, i.e., it differentiates telomerase from non-telomerase.

Other substitutions include replacing L-amino acids with the corresponding D-amino

25 acids. This rationale, moreover can be combined with the foregoing conservative substitution rationales. For example, D-leucine may be substituted for L-isoleucine. In addition, these D-amino acid-containing peptides may be prepared which have an inverse sequence, relative to the

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native sequence. Hence, ILAKFLHWL becomes LWHLFKALI. Such "retro-inverso" peptides are expected to have improved properties, such as increased in vivo half-life. This translates into smaller doses and more economically viable production.

Some embodiments contemplate multimers of the foregoing peptides.

5 Multimers can contain multiple copies of the same peptide, or they can be mixed and matched. The multimers can be direct tandem repeats, and may contain short spacers sequences of amino acids (e.g., 2-5 residues) like Gly and/or Pro, or other suitable spacers. Multimers may be any length, but typically will be less than about 100 amino acids. Preferred multimers are less than about 60 amino acids and have between about 2 and 5 copies of

10 peptides of about 8 to about 12 amino acids long. Multimers may also comprise several different telomerase antigens.

The telomerase antigens may be glycosylated or partially glycosylated according to methods known in the art. They also can be modified with large molecular weight polymers, such as polyethylene glycols. In addition, lipid modifications are preferred

15 because they may facilitate the encapsulation or interaction of the derivative with liposomes. Exemplary lipid moieties useful for this purpose include, but are not limited to, palmitoyl, myristoyl, stearoyl and decanoyl groups or, more generally, any C_2 to C_{20} saturated, monounsaturated or polyunsaturated fatty acyl group.

For convenience in making chemical modifications, it is sometimes useful to

20 include in a telomerase antigen one or more amino acids having a side chain amenable to modification. A preferred amino acid is lysine, which may readily be modified at the ε-amino group. Side-chain carboxyls of aspartate and glutamate are readily modified, as are serine, threonine and tyrosine hydroxyl groups, the cysteine sulfhydryl group and the histidine amino group. The introduction of two cysteine residues, at spaced locations in a peptide antigen,

25 may serve to form a structural constraint through a disulfide bridge, which may improve binding to MHC molecules.

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Also illustrative of a telomerase antigen within the present invention is a non-peptide "mimetic," *i.e.*, a compound that mimics one or more functional characteristics of the telomerase antigen. Mimetics are generally water-soluble, resistant to proteolysis, and non-immunogenic. Conformationally restricted, cyclic organic peptides which mimic telomerase antigens can be produced in accordance with known methods described, for example, by Saragovi, *et al. Science* 253: 792 (1991).

Telomerase antigens may also be constructed as hybrids (and/or formulated as distinct molecules together in liposomes, as described below) with immune-stimulatory molecules, like cytokines and adjuvants. Interleukin-2 (IL-2) is one such cytokine. Other cytokines include GM-CSF, IL-12 and flt-3 ligand. Telomerase antigens may be made as fusion proteins with IL-2, for example, by recombinant DNA or chemical synthetic means, or they may made as chemical conjugates using bi-functional chemical linkers. It is anticipated that such chimeric proteins would possess an increased ability to generate a T-cell-specific response against telomerase. Adjuvants include monophosphoryl lipid A (MPLA), and derivatives thereof, which also may be attached to a telomerase antigen by conventional linkers. Other conventional immune stimulatory molecules include keyhole limpet hemocyanin (KLH).

2. Identification of Other Useful Antigens

It is of interest to identify additional, and especially small telomerase antigens, which would be expected to generate a more specific response, associated with a particular epitope for example. Moreover, these small antigens may be more economically produced.

It is advantageous to identify additional telomerase antigen and further to refine the T-cell antigenicity of telomerase, even down to the epitopic level. One classic method involves proteolytic treatment of the large antigen to derive smaller antigens. In addition, fragments of protein antigens can be produced by recombinant DNA techniques and

assayed to identify particular epitopes. Moreover, small peptides can be produced by *in vitro* synthetic methods and assayed.

As an alternative to the random approach of making parts of the intact antigen then assaying them, a more biologically relevant approach is possible. Specifically, since antigenic fragments which bind to MHC class I and/or class II molecules, especially class I molecules, are of particular importance, one exemplary approach is to isolate the MHC molecules themselves and then to isolate the peptides associated with them. For a general description of such a method, see PCT/US98/09288; Agrawal *et al.*, Int'l Immunol.10:1907-16 (1998); and Agrawal *et al.*, Cancer Res. 55:515-56 (1998).

In a typical method, either primary tumor cells or a cell line expressing the antigen of interest are provided. In addition, it will be recognized that phagocytic antigen presenting cells (or any APC), such as macrophages, may be fed large antigens (or portions thereof) and thus act as the starting material for these methods. The MHC class I or class II molecules can be isolated from these starting cells using known methods, such as antibody affinity (MHC-specific antibodies) and chromatographic techniques.

Isolated MHC molecules are then treated to release bound peptides. This may be accomplished by treatment with agents that disrupt the interactions between the bound peptide and the MHC molecule, for example, detergent, urea, guanidinium chloride, divalent cations, various salts and extremes in pH. The peptides released can be further purified using conventional chromatographic and antibody affinity (using antigen-specific antibody) methodologies. The purified peptides may then be subjected to sequence and structural determinations, using for example peptide sequencing, gas chromatography and/or mass spectroscopy.

In this manner the sequences/structures of the most prevalent peptide epitopes associated with class I and/or class II molecules may be determined. Supplied with this sequence/structural information, permutations of the determined sequence can be made, as

detailed above, and assayed using known T-cell assays. Rammensee et al., supra, provides extensive methods and guidance related to identifying both class I and class II motifs.

Yet another method of generating telomerase antigens may utilize algorithms known in the art for predicting binding sequences. Publicly available comparison programs

5 using these algorithms to compare known peptide sequences to different HLA-binding motifs may be found, for example, at http://www-bimas.dcrt.nih.gov/hla_bind. Different class I and class II binding motifs may be found at that site or in publications like Rammensee et al. and Parker et al., both supra.

C. Vaccine Compositions

10 In general, any telomerase-specific antigen, as described above, will be useful in formulating telomerase-specific vaccines. Preferred antigens may be associated with lipids, usually either by direct lipid modification of the antigen and/or by liposomal association, as described below. The antigens may be administered as peptides or peptide mimetics, or they may be administered in nucleic acid form.

1. Liposomal Formulation

15 In one embodiment of the invention, the telomerase antigen is associated with a liposome. Techniques for preparation of liposomes and the formulation of various molecules, including peptides, with liposomes (e.g., encapsulation or complex formation) are well known to the skilled artisan. Liposomes are microscopic vesicles that consist of one or

20 more lipid bilayers surrounding aqueous compartments. See, generally, Bakker-Woudenberg et al. Eur. J. Clin. Microbiol. Infect. Dis. 12 (Suppl. 1): S61 (1993) and Kim, Drugs 46: 618 (1993). Liposomes are similar in composition to cellular membranes and as a result, liposomes generally can be administered safely and are biodegradable.

Depending on the method of preparation, liposomes may be unilamellar or

25 multilamellar, and can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes. Hydrophobic agents partition in

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the bilayers and hydrophilic agents partition within the inner aqueous space(s). See, for example, Machy et al., LIPOSOMES IN CELL BIOLOGY AND PHARMACOLOGY (John Libbey 1987), and Ostro et al., (1989) American J. Hosp. Pharm. 46: 1576.

Liposomes can adsorb to virtually any type of cell and then release the

5 encapsulated agent. Alternatively, the liposome fuses with the target cell, whereby the contents of the liposome empty into the target cell. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents. Scherphof et al., (1985) Ann. N.Y. Acad. Sci. 446: 368.

10 Anionic liposomal vectors have also been examined. These include pH sensitive liposomes which disrupt or fuse with the endosomal membrane following endocytosis and endosome acidification. Among liposome vectors, however, cationic liposomes are the most studied, due to their effectiveness in mediating mammalian cell transfection in vitro.

15 Cationic lipids are not found in nature and can be cytotoxic, as these complexes appear incompatible with the physiological environment in vivo which is rich in anionic molecules. Liposomes are preferentially phagocytosed into the reticuloendothelial system. However, the reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage

20 inactivation by pharmacological means. Classen et al., (1984) Biochim. Biophys. Acta 802: 428. In addition, incorporation of glycolipid- or polyethylene glycol-derivatised phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system. Allen et al., (1991) Biochim. Biophys. Acta 1068: 133; Allen et al., (1993) Biochim. Biophys. Acta 1150: 9.

25 Cationic liposome preparations can be made by conventional methodologies. See, for example. Felgner et al., Proc. Nat'l Acad. Sci USA 84:7413 (1987); Schreier, J. of

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Liposome Res. 2:145 (1992); Chang *et al.* (1988). *supra.* Commercial preparations, such as Lipofectin® (Life Technologies, Inc., Gaithersburg, Maryland USA), also are available. The amount of liposomes and the amount of DNA can be optimized for each cell type based on a dose response curve. Felgner *et al., supra.* For some recent reviews on methods employed

5 see Wassef *et al.*, Immunomethods 4: 217 - 222 (1994) and Weiner, A. L., Immunomethods 4: 217 - 222 (1994).

Other suitable liposomes that are used in the methods of the invention include multilamellar vesicles (MLV), oligolamellar vesicles (OLV), unilamellar vesicles (UV), small unilamellar vesicles (SUV), medium-sized unilamellar vesicles (MUV), large unilamellar

10 vesicles (LUV), giant unilamellar vesicles (GUV), multivesicular vesicles (MVV), single or oligolamellar vesicles made by reverse-phase evaporation method (REV), multilamellar vesicles made by the reverse-phase evaporation method (MLV-REV), stable plurilamellar vesicles (SPLV), frozen and thawed MLV (FATMLV), vesicles prepared by extrusion methods (VET), vesicles prepared by French press (FPV), vesicles prepared by fusion (FUV),

15 dehydration-rehydration vesicles (DRV), and bubblesomes (BSV). The skilled artisan will recognize that the techniques for preparing these liposomes are well known in the art. See COLLOIDAL DRUG DELIVERY SYSTEMS, vol. 66 (J. Kreuter, ed., Marcel Dekker, Inc. 1994).

2. *Suitable Adjuvants and Excipients*

The present vaccine formulations, liposomal or not, may be formulated

20 advantageously with some type of adjuvant. As conventionally known in the art, adjuvants are substances that act in conjunction with specific antigenic stimuli to enhance the specific response to the antigen. MPLA, for example, has been shown to serve as an effective adjuvant to cause increased presentation of liposomal antigen by the APCs to specific T Lymphocytes. Alving, C.R. 1993. *Immunobiol.* 187:430-446. Moreover, the skilled artisan

25 will recognize that other such adjuvants, such as Detox, alum, QS21, complete and/or incomplete Freund's adjuvant, MDP, LipidA and derivatives thereof, are also suitable.

Another class of adjuvants include stimulatory cyiokines, such as IL-2. Thus, the present vaccines may be formulated with IL-2 or IL-2 may be administered separately for optimal antigenic response. IL-2 is beneficially formulated with liposomes.

Vaccines may also be formulated with a pharmaceutically acceptable excipient.

5 Such excipients are well known in the art, but typically should be physiologically tolerable and inert or enhancing with respect to the vaccine properties of the inventive compositions. Examples include liquid vehicles such as sterile, physiological saline. When using an excipient, it may be added at any point in formulating the vaccine or it may be admixed with the completed vaccine composition.

10 Vaccines may be formulated for multiple routes of administration. Specifically preferred routes include intramuscular, percutaneous, subcutaneous, or intradermal injection, aerosol, oral or by a combination of these routes, at one time, or in a plurality of unit dosages. Administration of vaccines is well known and ultimately will depend upon the particular formulation and the judgement of the attending physician.

15 Vaccine formulations can be maintained as a suspension, or they may be lyophilized and hydrated later to generate a useable vaccine.

D. Targeting the Inventive Antigens and Vaccines

In order to provide greater specificity, thus reducing the risk of toxic or other unwanted effects during *in vivo* administration, it is advantageous to target the inventive

20 compositions to the cells through which they are designed to act, namely antigen-presenting cells. This may conveniently be accomplished using conventional targeting technology. One exemplary form of targeting using antibodies, or similar specifically-binding molecules, associated in some fashion with the antigen and/or vaccine composition.

Targeting molecules have the characteristic of being able to distinguish to

25 some degree, target APCs over background, non-APCs. Targeting molecules include mannose and the Fc portion of antibodies, and the like, which will target antigen presenting

cells. Targeting molecules may be directly associated with telomerase antigens, for example, by chemical conjugation or by fusion protein production, in the case of protein-based targeting sequences.

Due to their convenience and extensive familiarity in the art, antibodies and antibody derivatives are preferred targeting molecules. Antibodies and their derivatives include, but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies including single chain Fv (scFv) fragments, Fab fragments, F(ab')2 fragments, fragments produced by a Fab expression library, epitope-binding fragments, and humanized forms of any of the above. Of course, the smaller versions of these molecules are preferred, based on the fact that they will more readily target to an APC.

In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth et al., *J. Immunol. Methods* 35:1-21 (1980); Kohler and Milstein, *Nature* 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., *Immunology Today* 4:72 (1983); Cole et al., in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985), pp. 77-96). Affinity of the antisera for the antigen may be determined by preparing competitive binding curves, as described, for example, by Fisher, Chap. 42 in: *Manual of Clinical Immunology*: second edition, Rose and Friedman, eds., Amer. Soc. For Microbiology, Washington, D.C. (1980).

Fragments or derivatives of antibodies include any portion of the antibody which is capable of binding an APC target molecule, typically a surface antigen. Antibody fragments specifically include F(ab')2, Fab, Fab' and Fv fragments. These can be generated from any class of antibody, but typically are made from IgG or IgM. They may be made by conventional recombinant DNA techniques or, using the classical method, by proteolytic

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digestion with papain or pepsin. See CURRENT PROTOCOLS IN IMMUNOLOGY, chapter 2, Coligan et al., eds., (John Wiley & Sons 1991-92).

F(ab')2 fragments are typically about 110 kDa (IgG) or about 150 kDa (IgM) and contain two antigen-binding regions, joined at the hinge by disulfide bond(s). Virtually all, if not all, of the Fc is absent in these fragments. Fab' fragments are typically about 55 kDa (IgG) or about 75 kDa (IgM) and can be formed, for example, by reducing the disulfide bond(s) of an F(ab')2 fragment. The resulting free sulfhydryl group(s) may be used to conveniently conjugate Fab' fragments to other molecules, such as telomerase antigens or adjuvant molecules.

Fab fragments are monovalent and usually are about 50 kDa (from any source). Fab fragments include the light (L) and heavy (H) chain 'variable (V_L and V_H, respectively) and constant (C_L C_H, respectively) regions of the antigen-binding portion of the antibody. The H and L portions are linked by one or more intramolecular disulfide bridges.

Fv fragments are typically about 25 kDa (regardless of source) and contain the variable regions of both the light and heavy chains (V_L and V_H, respectively). Usually, the V_L and V_H chains are held together only by non-covalent interactions and, thus, they readily dissociate. They do, however, have the advantage of small size and they retain the same binding properties of the larger Fab fragments. Accordingly, methods have been developed to crosslink the V_L and V_H chains, using, for example, glutaraldehyde (or other chemical crosslinkers), intermolecular disulfide bonds (by incorporation of cysteines) and peptide linkers. The resulting Fv is now a single chain (*i.e.*, scFv).

Other antibody derivatives include single chain antibodies (U.S. Patent 4,946,778; Bird, *Science* 242:423-426 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., *Nature* 334:544-546 (1989)). Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain FV (scFv).

Derivatives also include "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci.*, 81:6851-6855 (1984); Neuberger et al., *Nature*, 312:604-608 (1984); Takeda et al.,

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Nature, 314:452-454 (1985)). These chimeras are made by splicing the DNA encoding a mouse antibody molecule of appropriate specificity with, for instance, DNA encoding a human antibody molecule of appropriate specificity. Thus, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a

5 variable region derived from a murine mAb and a human immunoglobulin constant region. Recombinant molecules having a human framework region and murine complementarity determining regions (CDRs) also are made using well-known techniques. These are also known sometimes as "humanized" antibodies and they and chimeric antibodies or antibody fragments offer the added advantage of at least partial shielding from the human immune

10 system. They are, therefore, particularly useful in therapeutic *in vivo* applications.

E. Nucleic Acid-Based Vaccines

Recently, there has been increased interest in polynucleotide-based vaccines, and such applications are contemplated here. These vaccines generally rely on either a DNA vector that encodes the antigen of interests under operable control of transcription and

15 translation signals, or a RNA vector that encodes the antigen of interests under operable control of translation signals. When these vaccines are administered, they are thought to be taken up by the surrounding cells, which then express the target antigen. The expressed antigen apparently becomes associated with the cell's major histocompatability (MHC) antigens and are thus localized to the surface of the cell and presented to immune cells. See,

20 for example, Corr *et al.*, J. Exp. Med. 184: 1555-60 (1996). Such vaccines may employ naked DNA (*Id.*) or the DNA may be liposomally associated or trapped (Gregoriadis *et al.*, FEBS Lett. 402: 107-10 (1997)).

These so-called "naked DNA" vaccines or vaccines comprising RNA have broad applicability. They may be employed. for example, as anti-cancer vaccines (Scheurs *et*

25 *al.*, Cancer Res. 58: 2509-14 (1998); Hurpin *et al.*, Vaccine 16: 208-15 (1998)) and anti-viral vaccines (Bohm *et al.*, Vaccine 16: 949-54 (1998); Lekutis *et al.*, J. Immunol. 158: 4471-77 (1997)). among others. Naked DNA vaccines have been shown to elicit both class I- (Scheurs

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. *et al.*, *supra*; Bohm *et al.* *supra*; Hurpin *et al.*, *supra*) and class II-restricted responses (Lekutis *et al.*, *supra*; Manickan *et al.*, J. Leukoc. Biol. 61: 125-32 (1997)).

Accordingly, any of the foregoing telomerase antigens may be administered as a naked DNA vaccine. These vaccines will comprise a nucleic acid vector that encodes a

5 telomerase antigen under the control of transcription and translation signals that operate in a mammal, preferably a human. They may be administered associated with or encapsulated by (usually cationic) liposomes, as detailed above, or they may be administered in any other physiologically tolerable excipient.

F. Methods of the Invention

10 According to one aspect of the invention, the foregoing telomerase antigens (or vaccine compositions) may be used as a conventional vaccine directly to induce an immune response against telomerase. In some cases. however, for an improved therapeutically or prophylactically suitable T-cell response, the antigen may be liposome-associated, as indicated above.

15 Since telomerase is expressed at high levels in cancer cells, the present antigens and vaccines are particularly suited for methods of treating and/or preventing cancer. A representative method involves administering to a cancer patient an effective amount of one or more of the foregoing telomerase antigens, which may be formulated as a vaccine. Again, smaller peptide antigens are preferred.

20 In addition. it is contemplated that the present telomerase antigens and vaccines will be particularly useful in *ex vivo* techniques. In general, these techniques entail isolating cells from a patient, contacting them with a telomerase antigen (or a vaccine, including nucleic acid vaccines) and administering the contacted cells back to the patient. In some cases (subject, for example, to MHC matching) the cells may be taken from one patient

25 for administration to another.

In one embodiment, autologous or compatible antigen presenting cells (usually dendritic cells or peripheral blood lymphocytes) are primed *ex vivo*, with a telomerase antigen. These "telomerase-primed" cells may then be transferred in beneficial amounts into a patient in need of therapy or prophylaxis. As with all aspects of the invention, the *ex vivo* priming step may be accomplished using lipid- and or liposome-associated small peptide antigen.

Yet another adoptive approach is contemplated. whereby antigen presenting cells are generated, as above, and used to generate autologous or compatible T-cells effectors *ex vivo*. T-cells so generated may be adoptively transferred in beneficial amounts to a patient in need. For a description of art-recognized techniques for adoptive T-cell transfer therapy, see Bartels, *et al.* Annals of Surgical Oncology. 3(1):67 (1996), which is hereby incorporated by reference.

Co-treatment with other immunostimmulatory, listed above, is also contemplated. Molecules like IL-2, GM-CSF. IL-12, flt-3 ligand, CD 40, and the like, are envisioned as quite useful. IL-2, for example, may be administered concurrently, separately or in a combined formulation, or it may be administered in an alternative dosing regime with the telomerase antigen or vaccine. In a one method, the IL-2 is formulated with liposomes.

In a further embodiment of the invention. any of the foregoing antigens or vaccines may be used in conjunction with known anti-cancer agents. One example includes MUC-1-based therapeutics. Numerous additional examples of these are well-known in the art Conventional chemotherapeutic agents include alkylating agents, antimetabolites, various natural products (*e.g.*, vinca alkaloids, epipodophyllotoxins, antibiotics, and amino acid-depleting enzymes), hormones and hormone antagonists. Specific classes of agents include nitrogen mustards, alkyl sulfonates, nitrosoureas. triazenes, folic acid analogues, pyrimidine analogues, purine analogs, platinum complexes. adrenocortical suppressants, adrenocorticosteroids, progestins, estrogens. antiestrogens and androgens. Some exemplary compounds include cyclophosphamide. chlorambucil. methotrexate, fluorouracil, cytarabine, thioguanine, vinblastine, vincristine, doxorubincin, daunorubicin, mitomycin, cisplatin,

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hydroxyurea, prednisone. hydroxyprogesterone caproate, medroxyprogesterone, megestrol acetate, diethyl stilbestrol. ethinyl estradiol. tomoxifen, testosterone propionate and fluoxymesterone.

A therapeutically or prophylactically beneficial or effective amount is an amount sufficient to induce a clinically relevant telomerase-specific T-cell response, as defined above. Clinical relevance can be determined by clinician.

Administration may be by any number of routes, including parenteral and oral. Cell-based vaccines are advantageously administered intravenously. Other vaccines typically will be administered intramuscularly, intradermally, subcutaneously or orally. The skilled artisan will recognize that the route of administration will vary depending on the nature of the vaccine formulation. Determining the optimal route of vaccination may be determined empirically and is well within the level of ordinary skill in the art.

Nucleic acid vaccines may also be administered by a variety of routes, the optimal route being determined empirically. For instance, some antigens have been found to elicit a superior cytotoxic response when administered intravenously. Hurpin *et al, supra*. For a superior immune response to oral administration, it may be advantageous to co-administer with the vaccine a mucosal adjuvant. like cholera toxin or cationic lipids. Ethchart *et al.*, J. Gen. Virol. 78: 1577-80 (1997). Intramuscular, intradermal and subcutaneous administration are also preferred.

EXAMPLES

EXAMPLE 1

This example illustrates the use of the telomerase-specific peptides to generate an antigen-specific cytotoxic T-cell response.

A. Materials and Methods

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In general, PCT/US98/09288; Agrawal et al., Int'l Immunol.10:1907-16 (1998); and Agrawal et al., Cancer Res. 55:515l-56 (1998) provide suitable methods, and those disclosures are hereby incorporated by reference, in their entirety.

Peptides. Peptides were selected using the HLA peptide search program at

5   http://www-bimas.dcrt.nih.gov. HLA-A2 specificity was selected, with default parameters. Three of the top twenty scores were synthesized and tested. These peptides has the sequences: RLVDDFLLV, ELLRSFFYV and ILAKFLHWL.

Preparation of Liposomes. The bulk liquid composition of liposomes consisted of dipalmitoyl phosphatidyl choline (DPPC), cholesterol (Chol) and dimyristoyl phosphatidyl

10   glycerol (DMPG) in a molar ratio of 3:1:0.25 and contained Lipid A at a concentration of 1% (w/w) of bulk lipid. Synthetic telomerase peptides were present in the aqueous phase during liposome formation at a concentration of 0.7 mg/mL, and approximately 28% of the input peptide was captured within the liposome structures. The formulated product contained 2 mg of bulk lipid, 20 μg Lipid A and about 20 μg of peptide per injected dose of 100 μl.

15   Bulk lipids and Lipid A were dissolved in chloroform/methanol (methanol was used initially to solubilize DMPG). The lipid mixture for each 4 ml preparation of liposomes consisted of 64 mg DPPC, 11 mg Chol, 5 mg DMPG, 0.8 mg Lipid A in 12 ml of chloroform/methanol in a molar ratio of 3:1:0.25 at a final lipid concentration of 30 mM. Each 12 ml of the lipid mixture was dried to a film by rotary evaporation at 53°C in a 250 ml round

20   bottom flask, and residual solvent was removed under high vacuum. The lipid film was hydrated by addition of 4 ml PBS containing the peptide and slow rotation of the flasks at 53°C followed by 5 cycles of vortexing and warming to 53°C.

Liposome structures were reformed to a more uniform size by a series of 5 freeze/thaw cycles consisting of freezing in a dry ice bath, thawing, warming to 41°C and

25   vortexing before beginning the next cycle. Liposomes then were collected by

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ultracentrifugation at 1500.000 x g at 4°C for 20 minutes, washed twice by addition of PBS and ultracentrifugation again. Liposomes were finally reconstituted to the desired volume.

Cytokines. In order to promote CTL generation, human recombinant cytokines, IL-12 (R&D Systems, Minneapolis, MN), IL-7 (Intermedico, Markham, Ontario)

5   were diluted in serum-free AIM-V media (Life Technologies) just prior to use.

General Procedures for Loading APCs with Liposome-encapsulated peptide. Human peripheral blood lymphocytes (PBLs) were purified from heparinized blood by centrifugation in Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). The Ficoll-blood interface layer obtained by centrifugation was collected and washed twice with RPMI before use.

10   Briefly, to $2\text{-}10 \times 10^6$ PBLs in 0.9 mL AIM-V media, one dose of liposome containing peptide formulation was added and the PBLs were incubated overnight at 37°C with CO_2 supplemented incubator. After incubation, the PBLs were treated with mitomycin C or γ-irradiated (3000 rads) followed by washing with AIM-V media.

Cytotoxic T lymphocyte assays. For the CTL assay, three (HLA-A2) normal

15   donors' PBLs were used. The T-cells were grown for five weeks in bulk cultures as described above. At the end of two weeks, live T-cells were harvested from flasks and counted. The targets were mutant T2 cells. Houbiers et al., Eur. J. Immunol 23:2072-2077 (1993); Stauss et al., Proc. Natl. Acad. Sci. U.S.A. 89:7871-7875 (1992). The telomerase peptide-mediated upregulation of HLA-A2 expression on T2 cells was examined using the HLA-A2-associated

20   peptides ILAKFLHWL (BP1-187), RLVDDFLLV (BP1-190), and ELLRSFFYV (BP1-191) using known methods. Townsend et al., Nature 346:476 (1989). T2 cells were loaded overnight at 37°C in 7% CO_2 with various the telomerase synthetic peptides at 200 μM in presence of 8 μg exogenous β2 microglobulin. Houbiers et al., supra; Stauss et al., supra. The peptide-loaded T2 target cells were loaded with ^{51}Cr (using $NaCrO_4$) for 90 minutes and

25   washed. CTL assays were performed as previously described. Agrawal et al., J. Immunol. 156:2089 (1996). Percent specific killing was calculated as: experimental release -

spontaneous release/maximum release - spontaneous release x 100. The effector versus target ratios used were 50:1, 25:1, 10:1 and 5:1. Each group was set up in four replicate and mean percent specific killing was calculated.

Cell Surface Immunofluorescence Staining. For detection of cell surface

5 antigens, the peptide-fed T2 cells were washed once in cold PBS containing 1% BSA followed by addition of 1 μg of anti-A2 monoclonal antibody, MA2.1 or a control antibody and incubated for 45 minutes on ice. Cells were then washed and a secondary antibody, goat anti-mouse IgG (H+L)-FITC labeled (Southern Biotech) was added for 30 minutes on ice.

B.   Results

10   Using the foregoing methods, the cytotoxic activity of T-cells stimulated with autologous APCs pulsed with liposomal telomerase peptide was determined. The source of T-cells was PBLs from three HLA.A2[+] donors. Target T2 cells (HLA.A2[+]) were loaded with the telomerase peptide indicated above.

The negative control was T2 cells, and the positive control was the 10-mer flu

15 peptide FLPSDYFPSV, which strongly upregulates HLA.A2 expression on T2 cells. These data confirm that the present methods can be used to generate specific, biologically relevant T-cell responses to telomerase, such as cytotoxicity.

Figure 1 shows FACS analysis of cells treated according to the foregoing methods, panel A shows the negative control. Panel B, is the positive control, showing a

20 227% increase in median channel intensity of A2 expression as compared to the negative control. Panel C, is the experimental with BP1-187, and shows a 396% increase in median channel intensity of A2 expression as compared to the negative control. Panels D and E show the results with BP1-190 and BP1-191, respectively yielding 0% and 6% increases over the negative control.

25   Table 1 shows specific killing of targets by telomerase antigen-primed (BP1-187) cytotoxic T-cells. The negative control was SIINFEKL.

These data indicate that, contrary to expectations, an immune response can be generated against telomerase, a self-antigen.

Table 1: Telomerase Peptide-Specific Killing

TARGET	EFFECTOR:TARGET RATIO	PERCENT KILLING
Unloaded	50:1	35.7
Unloaded	25:1	23.8
Unloaded	10:1	13.1
Unloaded	5:1	5.0
Telomerase Peptide-Loaded	50:1	56.7
Telomerase Peptide-Loaded	25:1	33.5
Telomerase Peptide-Loaded	10:1	21.1
Telomerase Peptide-Loaded	5:1	15.5
Control Peptide-Loaded	50:1	32.3
Control Peptide-Loaded	25:1	22.1
Control Peptide-Loaded	10:1	11.3
Control Peptide-Loaded	5:1	7.6

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    The foregoing detailed description and examples are presented for illustrative

5 purposes only and are not meant to be limiting. Further embodiments of the invention will be ready apparent to the skilled worker in view of this disclosure.

What Is Claimed Is:

1.   A peptide, comprising less than about 60 amino acids of the native telomerase protein sequence, optionally having one or more conservative substitutions, wherein said peptide is capable of binding at least one human leukocyte antigen (HLA).

2.   A peptide according to claim 1, wherein said HLA molecule is a class 1 molecule.

3.   A peptide according to claims 1 or 2 which is about 8 to about 12 amino acids in length.

4.   A peptide according to any of claims 1, 2 or 3, comprising the sequence ILAKFLHWL, or a conservative variant thereof.

5.   A vaccine, comprising at least a portion of the native telomerase protein sequence, optionally having one or more conservative amino acid substitutions, or a polynucleotide encoding said telomerase portion, and a lipid, wherein said telomerase portion is capable of binding at least one human leukocyte antigen (HLA).

6.   A vaccine according to claim 5, wherein said telomerase portion is a peptide of less than about 60 amino acids in length.

7.   A vaccine according to claims 5 or 6, wherein said telomerase portion is a peptide of about 8 to about 12 amino acids in length.

8.   A vaccine according to any of claims 5, 6 or 7, wherein said lipid is part of a liposome.

9.   A method of treating or preventing cancer, comprising administering to a patient in need thereof an effective amount of a composition which comprises at least a portion of the native telomerase protein sequence, optionally having one or more conservative

amino acid substitutions, or a polynucleotide encoding said telomerase portion, wherein said portion is capable of binding at least one human leukocyte antigen (HLA).

10. A method according to claim 9, wherein said composition further comprises a lipid.

11. A method according to claims 9 or 10, wherein said telomerase portion is a peptide of less than about 60 amino acids in length.

12. A method according to any of claims 9, 10 or 11, wherein said telomerase portion is a peptide of about 8 to about 12 amino acids in length.

13. A method according to any of claims 10, 11 or 12, wherein said lipid is part of a liposome.

14. A method of treating or preventing cancer, comprising administering to a patient in need thereof a telomerase-primed antigen-presenting cell.

15. A method according to claim 14, wherein said antigen-presenting cell is primed using a composition comprising at least a portion of the native telomerase protein sequence, optionally having one or more conservative amino acid substitutions, or a polynucleotide encoding said telomerase portion. wherein said telomerase portion is capable of binding at least one human leukocyte antigen (HLA).

16. A method according to claim 15. wherein said telomerase portion is a peptide of less than about 60 amino acids in length.

17. A method according to claim 16. wherein said telomerase portion is a peptide of about 8 to about 12 amino acids in length.

18. A method according to any of claims 15, 16 or 17, wherein the composition further comprises a lipid.

19. A method according to claim 18, wherein said lipid is in a liposome.

20. A method according to any of claims 14-19, further comprising administering an effective amount of interleukin-2 (IL-2) to said patient.

21. An isolated polynucleotide that encodes a telomerase specific antigen, wherein said polynucleotide is less than about 180 nucleotides in length.

22. A polynucleotide according to claim 21 which is about 24 to about 36 nucleotides in length.

23. A polynucleotide according to claims 21 or 22, wherein said antigen is a class 1-specific antigen.

24. A polynucleotide according to any of claims 21, 22 or 23, which encodes a peptide comprising the sequence ILAKFLHWL, or a conservative variant thereof.

25. A method of producing a telomerase-primed antigen-presenting cell, comprising contacting an antigen-presenting cell with a composition comprising at least a portion of the native telomerase protein sequence, optionally having one or more conservative amino acid substitutions, or a polynucleotide encoding said telomerase portion, wherein said telomerase portion is capable of binding at least one human leukocyte antigen (HLA).

26. A method according to claim 25, wherein said telomerase portion is a peptide of less than about 60 amino acids in length.

27. A telomerase-primed antigen-presenting cell which is produced according to claim 25 or 26.

28. A peptide according to claim 2 which is less than about 25 amino acids in length.

29. A polynucleotide according to claim 21 which is less than 75 nucleotides in length.

30. Use of a composition comprising at least a portion of the native telomerase protein sequence, optionally having one or more conservative amino acid substitutions, or a polynucleotide encoding said telomerase portion, wherein said telomerase portion is capable of binding at least one human leukocyte antigen (HLA), for treating or preventing cancer.

31. A use according to claim 30, wherein said composition further comprises a lipid.

32. A use according to claim 31, wherein said lipid is part of a liposome.

33. A use according to any of claims 30-32, wherein said telomerase portion is a peptide of less than about 60 amino acids in length.

34. A use according to any of claims 30-33, wherein said telomerase portion is a peptide of about 8 to about 12 amino acids in length.

35. Use of a telomerase-primed antigen-presenting cell for treating or preventing cancer.

36. A use according to claim 35, wherein the antigen-presenting cell is primed using a composition comprising at least a portion of the native telomerase protein sequence, optionally having one or more conservative amino acid substitutions, or a polynucleotide encoding said telomerase portion, wherein said telomerase portion is capable of binding at least one human leukocyte antigen (HLA).

37. A use according to claim 36, wherein said telomerase portion is a peptide of less than about 60 amino acids in length.

38. A use according to claims 36 or 37, wherein said telomerase portion is a peptide of about 8 to about 12 amino acids in length.

39. A use according to any of claims 36-38, wherein the composition further comprises a lipid.

40. A use according to claim 39, wherein said lipid is in a liposome.

41. Use of a composition comprising a telomerase-primed antigen-presenting cell according to any of claims 15-19, wherein the composition further comprises interleukin-2 (IL-2), for treating or preventing cancer.
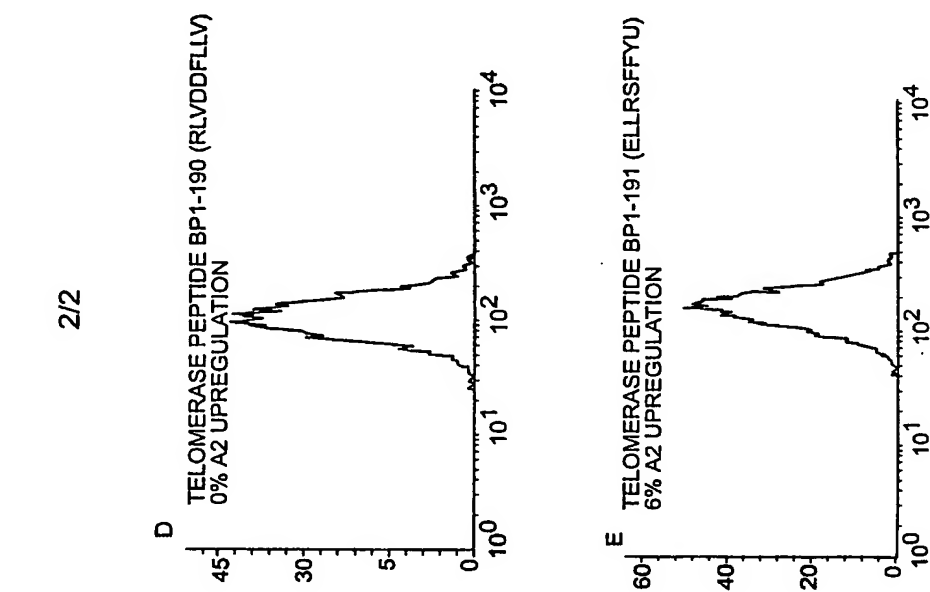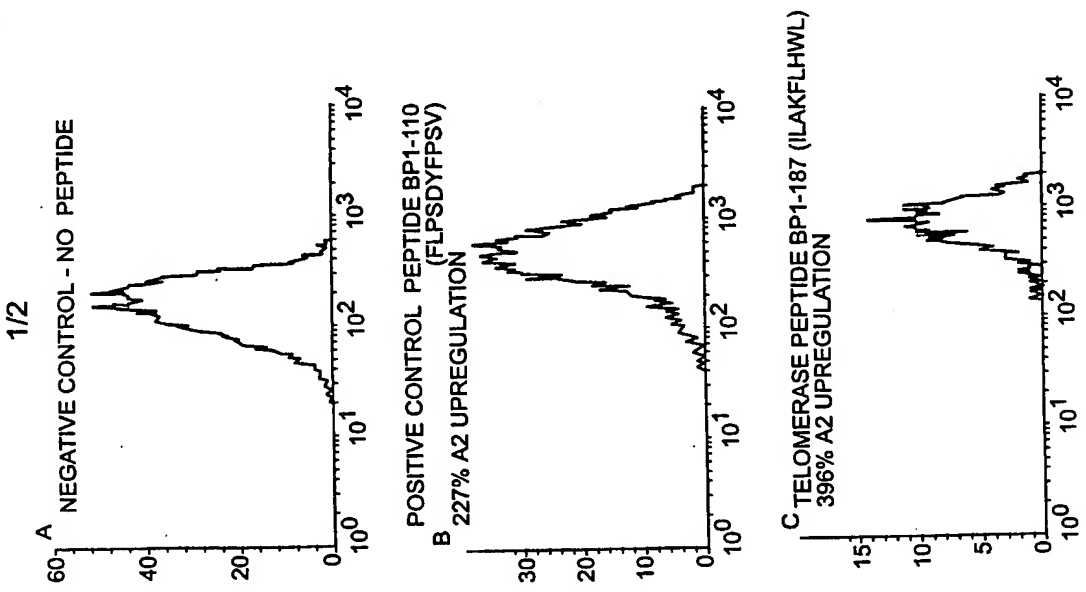
2/2

D TELOMERASE PEPTIDE BP1-190 (RLVDDFLLV)
0% A2 UPREGULATION

E TELOMERASE PEPTIDE BP1-191 (ELLRSFFYU)
6% A2 UPREGULATION

FIG. 1 CONT.

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1/2

A NEGATIVE CONTROL - NO PEPTIDE

B POSITIVE CONTROL PEPTIDE BP1-110
(FLPSDYFPSV)
227% A2 UPREGULATION

C TELOMERASE PEPTIDE BP1-187 (ILAKFLHWL)
396% A2 UPREGULATION

FIG. 1

SUBSTITUTE SHEET (RULE 26)